Multimer Polynucleotide Synthesis

[1] The present invention relates to a process for the preparation of polynucleotides, whereby under suitable conditions the free 5′-hydroxy group of selected oligonucleotides, whose terminal 3'- hydroxy group contains a usual suitable protecting group, is reacted with a hydroxy group, derivatized in a previous reaction step to a phosphite amidoester or to a phosphonic acid ester, whereby said hydroxy group is a 3'-hydroxy function of a solid - phase bound polynucleotide, or a solid phase bound hydroxy function.

[2] Further the present invention relates to a kit for performing a process, according to the invention, which contains at least one or more selected oligonucleotide(s) having a free 5'-hydroxy group and a protected 3'-hydroxy group.

[3] Further the present invention relates to the use of the process or kits, according to the invention, for the preparation of oligonucleotides or nucleic acid chips.

[4] Synthetic oligonucleotides are used in all areas of gene technology, for example in gene transfection or in gene analysis. Polynucleotides are prepared by chain extension of a starting compound with many individual nucleoside building blocks. For the synthesis the reacting hydroxy groups are derivatized in such a manner as to form a phosphodiester group, a phosphotriester group or an H-phosphonate group. Other functional groups of the starting compounds, interfering with this reaction, will have usual suitable protecting groups.

[5] For instance DE 199 15 867 A1 and DE 199 38 092 A1 describe photolabile protecting groups for hydroxy groups, which can be introduced into a nucleoside or nucleotide with high yields and release the protected hydroxy group when irradiated.

[6] Nowadays, polynucleotides are prepared primarily by using solid phase techniques in order to optimize the efficiency of the process. The starting compounds are bound either directly or via linkers to functionalized solid surfaces of polymer beads or to glass-, metal- or plastic surfaces and reacted with reagents required for extending polynucleotide chains.

[7] Excess reagents as well as soluble reaction byproducts and solvents can easily be removed from the solid phase bound polynucleotide compounds.

[8] A disadvantage of the known processs is that the plurality of the individual reaction steps lead to low overall yield, even when their individual yield is high.

[9] Therefore, depending on the desired length of the nucleotide chain and the number of individual reaction steps, an excess of starting compounds and reagents has to be used, which require after completed reaction a complex process for (possibly ineffective) re-use. Moreover numerous and often similar undesired byproducts have to be separated from the end product.

[10] Thus a person skilled in the art has to use significant amounts of starting compounds and has to perform complex purification processes.

[11] The problem of the present invention is to provide a process, which does not have the disadvantages of the current state of the art. Such a process in particular should be suitable for automated solid phase synthesis of polynucleotides.

[12] The problem is solved by a process for the preparation of polynucleotides comprising the following steps:

a) The reaction of the free 5′-hydroxy group of a selected oligonucleotide, whose terminal 3′-hydroxy group contains a usual suitable protecting group, with a hydroxy group derivatized in a preceeding reaction step to a phosphite amidoester or to a phosphoric acid ester or to an H-phosphonate, whereby said hydroxy group is a 3'-hydroxy group of a free or solid phase bound polynucleotide or a solid phase bound hydroxy group under suitable conditions and if necessary with the purification of the reaction product,

b) where applicable the oxidation of the reaction product to a phosphodi- or phosphotriester according to step a), if a hydroxy group was used, which has been derivatized to a phosphite amidoester or an H-phosphonate and purification of the reaction product if necessary.

c) Removal of the 3'-hydroxy protecting group of the reaction product according steps a) or b) under suitable conditions and purification of the reaction product if necessary.

d) Where necessary, derivatization of the released 3'-hydroxy group to a phosphite amidoester or in an H-phosphonate according to step c) by using the appropriate usual reagents.

e) if necessary rerun steps a) to c) by using the reaction product activated according step d),

[13] whereby the oligonucleotides with the free 5′-hydroxy function according to step a) are selected in such a way that the desired polynucleotide is obtained.

[14] In the following by "suitable conditions" and "usual suitable conditions" those reaction conditions are understood, which are familiar to the person skilled in the art, as e.g. solvents, temperature, catalyst, energy input (thermally or by radiation), which result in a high coupling resp. cleavage yield.

[15] A preferred embodiment of the present invention comprises steps a) to e) of the above described process, where the free hydroxy group or the 3'-hydroxy group of the polynucleotide is present as solid phase phosphite amidoester (or phosphoric acid ester) and is reacted with the free 5'-hydroxy group of a selected oligonucleotide.

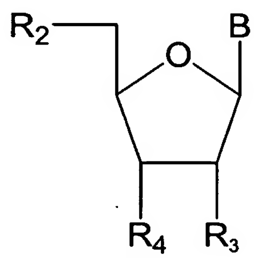
[16] By using selected already functionalized oligonucleotides and starting compounds, which contain free and/or derivatized reactive hydroxy groups, intermediate steps are avoided, as they are necessary for polynucleotide chain extensions with individual nucleotides. For instance with selected dinucleotides only 1/2 of the necessary couplings are required, with trinucleotides only 1/3 of the necessary couplings are required and so forth.

[17] The selected oligonucleotides, as understood according to the invention, are polynucleotides with 2 to 10 nucleosides, which are preferably connected to each other via 3'-5'- phosphoric acid ester bonds. Polynucleotides also comprise oligonucleotides and polynucleotides with more than 10 nucleotide building blocks.

[18] Selected oligonucleotides, used according to the invention, are pentanucleotides, preferably tetranucleotides, especially preferred trinucleotides and exceptionally preferred dinucleotides.

[19] The process is also suitable for the preparation of extra long polynucleotides according to the so-called "block condensation process", resp. for preparation of large quantities of polynucleotides according to the block condensation process, since unreacted educts can easily be retrieved and reused in later syntheses or synthesis steps.

[20] The selected oligonucleotides can for instance be composed of nucleoside building blocks according to formula (X), which are connected via 3'-5'- phosphoric acid ester bonds:



(X)

[21] where B can be an H, adeninyl, cytosinyl, guaninyl, thyminyl, uracilyl, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosin-1-yl, 5-amino-4-carboxylimidazol-1-yl or 5-amino-4-carbamoylimidazol-1-yl, whereby existing primary amino functions can be protected by a permanent protecting group, resp. thyminyl or uracilyl at the O_4-position can contain a permanent protecting group,

[22] and where R_2 can be a phosphoric acid ester rest, a free hydroxy group, a phosphite amidoester, a phosphonic acid rest or another suitable hydroxy protecting group,

[23] R_3 can be an H, OH, halogen, acylamino-, alkoxy- or alkoxyalkyl rest with 1 to 4 C-atoms,

[24] R_4 can be a phosphoric acid rest, a free hydroxy group, a phosphite amidoester rest, a phosphoric acid ester rest, an H-phosphonate rest or a hydroxy protecting group.

[25] The synthesis of selected oligonucleotides can be performed for instance according to Figure 1a. Even though this leads to a dinucleotide, it is clear that also tri-, tetra-, penta- and even higher nucleotides can be prepared this way.

[26] In step I the free 3′-hydroxy function of a nucleoside compound A, which has a nucleobase B_2 and whose 5′-hydroxy function is protected by a dimethoxytrityl protecting group, can be derivatized with NPPOC-chloride and thus be protected.

[27] In step II the dimethoxytrityl protecting group (DMT) of the compound B, which thus has been prepared, is cleaved under acid conditions and the 5′-hydroxy function is released.

[28] In step III the free 5′-hydroxy function of compound C is reacted with the 3'-hydroxy function of the nucleoside D, which had been previously derivatized to a phosphite amidoester (R_1 can be for instance C_1 to C_4). In compound C in Fig. 1a L stands for NPPOC, FMOC and NPC. Nucleoside D has a dimethoxytrityl-protecting group (DMT) at the 5′-end and a base B_1. After oxidation of the phosphite ester bond into a phosphate ester bond and after cleavage of the 5'-terminal DMT-group, the resulting dinucleotide E, whereby L stands for NPPOC, FMOC and NPC, can now directly be used as selected oligonucleotide in a process according to the invention. Other dinucleotides or oligonucleotides with other bases are available by selecting the corresponding starting compounds C and D.

[29] A dinucleotide E can also be transformed into other long chain oligonucleotides by repeated transformation with nucleosides of e.g. compound D. Further a dinucleotide E can also be transformed with other oligonucleotides into new oligonucleotides or polynucleotides, whose terminal 3′and 5′-hydroxy group are derivatized equally or functionally equal as compound D.

[30] Another synthesis for polynucleotides according to the invention is shown in Figure 1b, where instead of a NPPOC- protecting group in 3'-position a p-nitrophenyloxycarbonyl-protecting group is used, whereby Y = O,S . After cleavage of the DMT-protecting group in 5'-position under acidic conditions and reacting with the phosphite amidoester-derivatized mononucleoside (D), an oxidation of the phosphite ester bond into the phosphate ester is performed.

[31] With the reaction scheme according to Figure 1b, as opposed to Figure 1a, the DMT-protecting group in 5'-position is cleave only after completed reaction with (J), as it is described in the following in more detail.

[32] For the preparation of polynucleotides, compound (J) in contrast to compound (E), is reacted with the compound XI below only after completed reaction, while being activated with DMAP,

[33] whereby the methyl group can also be an H, and wherein X = O,S or HNR_3 , and where R_3 = H, alkyl, aryl or aralkyl , in the process according to the invention and after cleavage of the 5'-DMT-protecting group. In Fig. 1b, compound IX is represented but not limited to NPPOH.

[34] A subsequent cleavage of the DMT group under acidic conditions leads to compound (N).

[35] However, all protecting groups commonly used by a person skilled in the art are suitable as intermediary protecting groups of the 3'-hydroxy function. These are protecting groups, which are orthogonal to DMT and cleavable from the permanent base protecting groups, especially photolabile protecting groups.

[36] Preferred photolabile protecting groups of the 3'-hydroxy function are NPPOC, MeNPOC, NPES, NPPS, PyMOC, NVOC, and NBOC. Reagents like e.g. the corresponding chlorides or alcohol are used analogously for the introduction of these protecting groups.

[37] For example as described in Figure 2, derivatized hydroxy functions of the type phosphite amidoester F (or D) or H-phosphonate resp. H-phosphonate salts (K) or phosphoric acid ester G, whereby A is a halogene selected from F, Cl, Br and R and where R can be an H, alkyl, cycloalkyl, aryl, aralkyl, haloalkyl, cyanoalkyl rest, and if R = H , the compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt and n = 0 or an integer from 1 to 4, are used for the preparation of polynucleotides according to the invention.

[38] In Fig. 2 X represents an example of a nucleoside or nucleotide rest, a oligo- or polynucleotide rest, a solid phase linker, a hydroxylic derivatized solid phase surface or other possible compounds, which contain a hydroxyl group. Such starting compounds can be freely soluble or bound to solid phases.

[39] The starting compounds can be either soluble or solid phase bound nucleosides or polynucleotides, whose terminal 3'-hydroxy function is a phosphite amidoester, a phosphonic acid ester or an H-phosphonate. Also hydroxy functions of the solid phase itself or its linkers can serve as starting compound, in the form of phosphite amidoester derivatives or phosphoric acid derivatives (G) or H-phosphonates (K).

[40] Besides the selected oligonucleotides also selected mononucleosides can be used in a supplementary way or predominantly, in order to obtain the desired nucleotide chain, if one or another of the necessary oligonucleotides is not available.

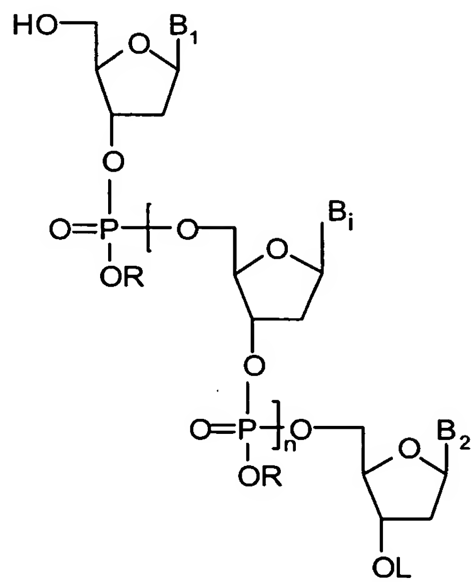
[41] In this case the starting compounds derivatized as phosphite amidoester (F) or phosphoric acid ester (G) resp. (K) can then react with a selected oligonucleotide, for instance compound (E) (Figure 2) by forming the desired polynucleotide in steps IV, Va and Vb. In compound E in Fig. 2 L stands for NPPOC, FMOC and NPC. The phosphite amidoester must be activated with 1-H-tetrazol (TET) or 4,5-dicyanoimidazol (DCI) in acetonitrile before the reaction. The H-phosphonate salt (K) is activated before the reaction Vb with pivaloylchloride or adamantoylchloride in triethylamine/acetonitrile. The coupling product is either the end product or an intermediary product, which still has to be extended.

[42] If the desired end product has already been obtained, just the protecting groups at the terminal 3'- end, here a NPPOC- protecting group, as well as all so-called permanent protecting groups have to be cleaved, if necessary after oxidation of the trivalent phosphorus (Figure 2, step VI).

[43] If the elongated intermediary product (E) shall be extended even further, the deprotected 3'-hydroxy group is derivatized again in step VII to form the phosphite amidoester H or the corresponding phosphonic acid ester and is reacted with another oligonucleotide E. For this the NPPOC- protecting group must first be transformed into a hydroxy function (Step VI). This reaction sequence is repeated, by varying the selected oligomers and if necessary some individual nucleosides (derivatized correspondingly), as often as necessary until the desired polynucleotide is obtained (step VIII).

[44] Furthermore the problem of the present invention is solved by providing new nucleotide derivatives (L), (E), (M) and (J). These are preferably used as building blocks in the described process according to the invention.

[45] The nucleotide derivative (L) has the following general formula,

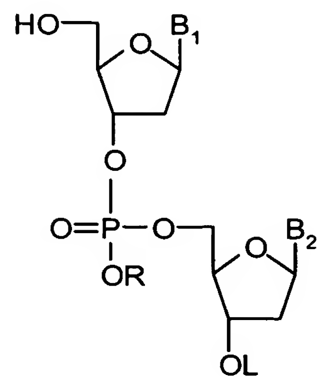


(L)

[46] where B_1, B_2, B_i can be H, adeninyl, cytosinyl, guaninyl, thyminyl, uracilyl, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosin-1-yl, 5-amino-4-carboxylimidazol-1-yl or 5-amino-4-carbamoylimidazol-1-yl, independently from each other, and in the case of B_1, B_2, B_i, where a primary amino function is present, can have a permanent protecting group resp. with thyminyl or uracilyl at the O_4-position can have a permanent protecting group if necessary,

[47] and where R can be an H, alkyl, cycloalkyl, aryl, aralkyl, haloalkyl, cyanoalkyl rest, and if R = H , the compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt and n = 0 or an integer from 1 to 4, and where L stands for NPPOC, FMOC and NPC.

[48] The nucleotide derivative (E) has the following general formula:

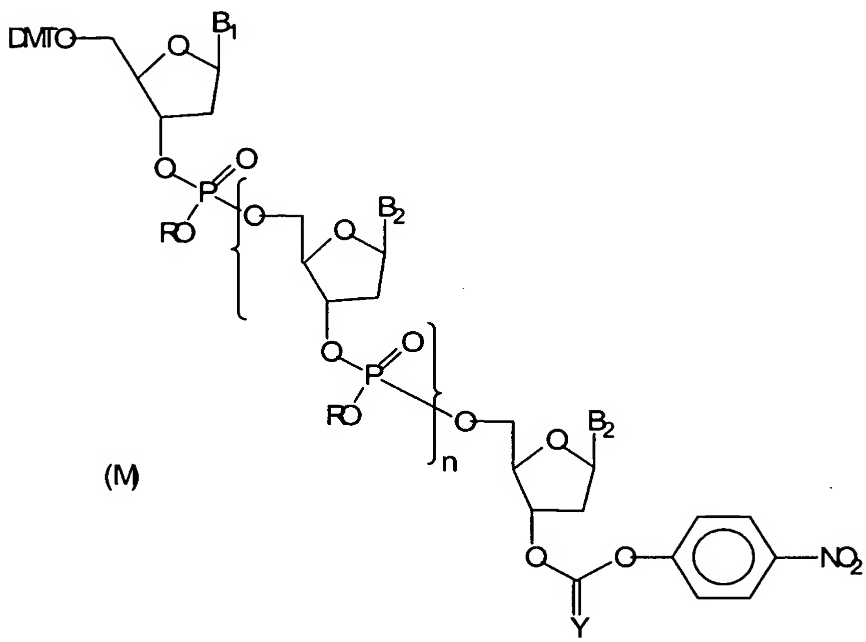


(E)

[49] where B_1 and B_2 can be H, adeninyl, cytosinyl, guaninyl, thyminyl, uracilyl, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosin-1-yl, 5-amino-4-carboxylimidazol-1-yl or 5-amino-4-carbamoylimidazol-1-yl independently from each other, and in the case of B_1, B_2, where primary amino functions are present, can have a permanent protecting group resp. with thyminyl or uracilyl at the O_4-position can have a permanent protecting group if necessary,

[50] and where R can be an H, alkyl, cycloalkyl, aryl, aralkyl, haloalkyl, cyanoalkyl rest. If R = H, the compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt, and where L stands for NPPOC, FMOC and NPC.

[51] The nucleotide derivative (M) according to the invention has the following formula:



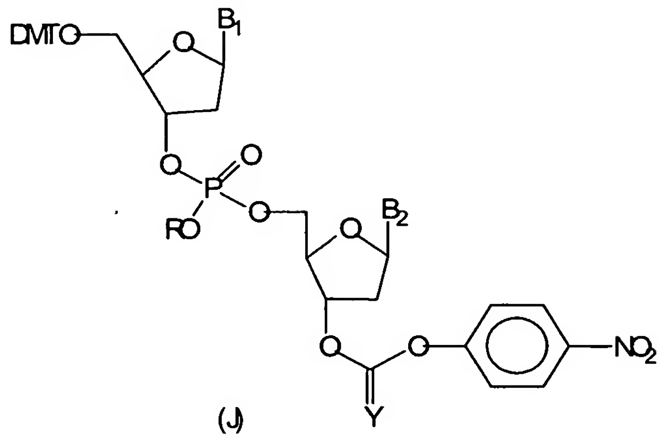
(M)

[52] where B_1, B_2, B_i can be H, adeninyl, cytosinyl, guaninyl, thyminyl, uracilyl, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosin-1-yl, 5-amino-4-carboxylimidazol-1-yl or 5-amino-4-carbamoylimidazol-1-yl independently from each other, and in the case of B_1, B_2, B_i , where primary amino functions are present, can have a permanent protecting group resp. with thyminyl or uracilyl at the O_4-position can have a permanent protecting group if necessary. If R = H, the present compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt.

[53] and where R can be an H, alkyl, cycloalkyl, aryl, aralkyl, cyanoalkyl, haloalkyl rest. If R = H, the compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt.

[54] and Y = O or S and n = 0 or an integer from 1 to 4.

[55] The nucleotide derivative (J) has the following formula:



(J)

[56] where B_1 and B_2 can be H, adeninyl, cytosinyl, guaninyl, thyminyl, uracilyl, 2,6-diaminopurin-9-yl, hypoxanthin-9-yl, 5-methylcytosin-1-yl, 5-amino-4-carboxylimidazol-1-yl or 5-amino-4-carbamoylimidazol-1-yl independently from each other, and in the case of B_1, B_2, B_i, where primary amino functions are present, can have a permanent protecting group resp. with thyminyl or uracilyl at the O_4-position can have a permanent protecting group if necessary,

[57] R can be an H, alkyl, cycloalkyl, aryl, aralkyl, haloalkyl, or cyanoalkyl rest. If R = H, the present compound is preferably a soluble phosphorus diester salt, resp. in the form of a quarternary ammonium salt.

[58] and Y = O or S.

[59] The use of a p-nitrophenyl-O-C(Y)- protecting group advantageously facilitates to avoid the highly toxic and dangerous phosgene during introduction of protecting groups.

[60] The use of the dinucleotides (E) and (J) according to the invention resp. of the oligonucleotides (L) and (M) according to the invention, in the process according to the invention permits a fast and specific synthesis of long polynucleotides. At the same time higher selectivity and higher yield are obtained, since intermediary steps, as they weren necessary, with the previous processes according to the state of the art with the use of mononucleotides do not apply.

[61] The building blocks needed for chain extension are more stable and have a longer shelf life than building blocks of the state of the art and can be recovered after the reaction, as the activation takes place at the solid support in contrast to the state of the art, where the incoming building block, which is usually present in a 2 to 9 fold molar excess over the growing oligonucleotide, is activated to a highly reactive yet unstable intermediate, the excess being discarded after the coupling step. As the compounds of the invention are not activated, any excess material can be reused in subsequent syntheses or synthesis steps even without further purification. The so-called capping step does not apply, since the 3'-hydroxy functions, directly transformed into phosporus III, in subsequent synthesis steps, after activation but without completed coupling, cannot be extended. The reaction therefore substitutes the capping step completely. This is the case above all, because the reactivity of the reagents used for phosphitization is higher than that of the so-called capping reagents.

[62] The compounds, which have a hydroxy function derivatized as phosphite amidoester or phosphonic acid ester, are preferably bound to a solid phase.

[63] Preferred solid phases are carrier materials made from silica gel, glass, metal, preferably magnetic metal, plastic, cellulose, dextrane cross-linked with epichlorohydrine, agarose, styrene-divinylbenzene resins, preferably 4-(Hydroxymethyl)- phenoxymethyl-copolystyrene-divinylbenzene resins or chloromethylated Co-polystyrene-divinylbenzene resin, especially preferred styrene-divinylbenzene resins with 1 % divinylbenzene content.

[64] Plastic carrier materials comprise plastic films resp. membranes made of polypropylene, Nylon, cellulose, cellulose derivatives, for instance cellulose acetate, cellulose-mixed ester, polyether sulfones, polyamides, polyvinylchloride, polyvinyliden fluoride, polyester, Teflon or polyethylene.

[65] The carrier surface can contain free or protected functional groups, e.g. amino-, hydroxyl-, carboxyl-, carbonyl-, thiol-, amide- or phosphate groups. Such groups can also be connected with the polynucleotide via a linker molecule.

[66] Planar carrier surfaces are used as nucleic acid chips. Nucleic acid chips, according to the invention, are biomolecules built on a solid carrier, like DNA or RNA, and nucleic acid analogs, like PNA, LNA or chimeras of those with DNA, RNA or nucleic acid analogs.

[67] The process according to the invention is preferably used for the manufacture of nucleic acid chips, where the synthesized polynucleotide is attached to the solid phase via

the 5'-end and the 3'-OH group is freely accessible. Such nucleic acid chips are suitable both for hybridization experiments and for certain enzyme reactions (e.g. DNA-ligase, DNA-polymerase), that require a free 3′-OH. By using the process according to the invention nucleic acid chips are available, which are to be prepared faster, in higher yields and also containing longer polynucleotides than using the traditional nucleoside for nucleotide synthesis.

[68] The processes according to the invention are not only suitable for DNA- and RNA-nucleotide synthesis. The synthesis of polynucleotides made of nucleic acid analogs, like PNA, LNA or chimeras of those with DNA, RNA or nucleic acid analogs is also possible.

[69] The processes according to the invention are particularly suitable for implementation in an automated process. Such an automated process is preferably carried out as parallel synthesis for the preparation of a nucleotide library, where the selected oligonucleotides and if necessary some mononucleotides are selected specifically or at random.

[70] It is time saving and useful for the person skilled in the art to generate an oligonucleotide pool of different nucleic acid sequences for use in the process according to the invention. These different nucleic acid sequences should be preferably already derivatized correspondingly, so that they can be used without further processing.

[71] It is particularly advantageous that the unused portion of oligonucleotides can be reused in a subsequent synthesis step without further reconditioning. Furthermore the oligonucleotide building blocks are more stable as compared to the state of the art and therefore have a longer shelf life.

[72] The process according to the invention is also preferably used for large-scale manufacturing of therapeutic nucleic acid analogues in a cost-effective manner. Also, the reduction of the number of chemical unit-operations, like synthesis, washing and drying steps significantly lower the overall-cost of large scale oligonucleotide synthesis. Even more, according to the process, oxidation and cleavage reactions can be performed in one step (VII and VIII).

[73] Another specification of the present invention comprises a kit, which contains part of or all reagents and/or auxiliary supplies and/or solvents and/or work instructions for the implementation of a process according to the invention in one unit. The kit contains at least

one or several selected oligonucleotides, which preferably contain a free 5'-hydroxy function and a protected 3'-hydroxy function.

[74] Another embodiment of the present invention comprises the use of processes according to the invention and/or the above mentioned kit for the preparation of oligonucleotides or nucleic acid chips, preferably for the automated preparation of oligonucleotides or nucleic acid chips or nucleic acid analogues in a large scale.

[75] Abbreviations

DCI	4,5-dicyanoimidazole
DMT	dimethoxytrityl-
TsOH	toluenesulfonic acid
NPPOC	2-(2-nitrophenyl)propyloxycarbonyl
PYMOC	pyrenylmethyloxycarbonyl
MeNPOC	2-(3,4-methylendioxy-2-nitrophenyl)propyloxycarbonyl
NPE	4-nitrophenylethyl
NPC	4-nitrophenyloxycarbonyl
NPS	2-nitrophenylethylsulfonyl
NPPS	2-(2-nitrophenyl)propylsulfonyl
NVOC	2-nitroveratryloxycarbonyl
NBOC	2-nitrobenzyloxycarbonyl
NPPOH	2-(2-nitrophenyl)propanol
DMAP	4-(dimethylamino)-pyridine
FMOC	9-fluorenylmethoxycarbonyl
Bz	benzoyl

Figures

[76] Figures 1a and 1b show illustrative, non limiting examples of synthesis schemes for the preparation of selected oligonucleotides,

[77] Figure 2 shows an example of an illustrative non limiting synthesis scheme for the preparation of polynucleotides according to the invention using a process according to the invention,

[78] Figure 3 shows a further non-limiting synthesis scheme for oligonucleotide dimers according to the invention.

[79] Figures 1 and 2 are explained in detail in the foregoing description.

[80] Figure 3 shows another exemplary embodiment of the invention for the synthesis of oligonucleotides according to the invention, namely a number of 3′FMOC protected oligodinucleotides synthesized via the process according to the invention.

[81] In a first reaction step, the free 3′-hydroxy function of a nucleoside compound I, which has a nucleobase B, which is thyminyl (T) or benzoyl protected cytosinyl (C^{Bz}) and whose 5′-hydroxy function is protected by a dimethoxytrityl protecting group (DMT), is derivatized with FMOC-chloride and be protected. This reaction step leads to compounds 20 and 21. In the next reaction step, the dimethoxytrityl protecting group (DMT) of the compounds 20 and 21 is cleaved under acid conditions and the 5′-hydroxy function is released, yielding compounds 22 and 23. It is understood that a similar reaction falling under the scope of the invention can be carried out by using compound (J) and the corresponding alcohol like FMOH and the like. The reaction conditions are described in detail in the following examples, but are not limited to those set forth and may be applied and varied according to the needs of a person skilled in the art such applications and variations being also within the scope of the invention. This concerns especially reaction time, solvent, reactive agents, temperature pressure etc.

[82] The next reaction step of the reaction scheme involves the tetrazole-assisted coupling reaction of the free 5′-hydroxy function of compounds 22 or 23 with the 3'-hydroxy function of the nucleoside II, which had been previously derivatized to a phosphite amidoester. It is understood that any other coupling assisting agent known by a person skilled in the art can also be used. Nucleoside II has a dimethoxytrityl-protecting group (DMT) at the 5′-end and a base B_1. which is for example thyminyl (T) or benzoyl protected adeninyl (A^{Bz}). Coupling and oxidation with usual reagents known to a person skilled in the art, for example iodine, of the phosphite ester bond into a phosphate ester bond are performed as subsequent steps in a one-pot reaction leading to compounds 24 and 25. After

cleavage of the 5'-terminal DMT-group under acidic conditions, preferably with TsOH or other suitable acids, known by a person skilled in the art, the resulting dinucleotides 26 and 27 can now directly be used as selected oligonucleotide in a process according to the invention. Other dinucleotides or oligonucleotides with other bases are available by selecting the corresponding starting compounds. The reaction conditions for this specific reaction involving FMOC protected oligonucleotides are described in detail in the following examples 15 to 26, but are not limited to those set forth and may be applied and varied according to the needs of a person skilled in the art such applications and variations being also within the scope of the invention. This concerns especially reaction time, solvent, reactive agents, temperature pressure etc.

[83] In the following the present invention is further explained by a number of examples while making reference to the corresponding figures. These examples are meant solely for the explanation and illustration of the invention and do not restrict or limit the general idea underlying the invention.

Examples

Example 1

[84] *Preparation of 3-0-[2-(2-Nitrophenyl)propyloxycarbonyl]thymidine.*

[85] 4.05 g 2-(2-Nitrophenyl)propyloxycarbonylchloride (NPPOC-Cl) (16.6 mmol, 1.3 equivalents) in 5 ml dichloromethane were added to a solution of 7 g 5'-0-Dimethoxytrityl-thymidine (DMTr-Thd) (12. 8 mmol) in 70 ml pyridine under argon protection atmosphere, while being cooled in an ice-water bath. The ice bath was removed and the reaction mixture was stirred at room temperature for 4 hours. Subsequently 2 ml methanol was added to the reaction solution. After another 15 min the reaction mixture was diluted with 250 ml dichloromethane, and washed 2x with 100 ml water. The organic phase was dried over sodium sulfate and evaporated until an oil was obtained. The rest was evaporated with some added toluene until an oil was obtained.

[86] The resulting oil was dissolved in a mixture of dichloromethane and toluene (20+10 ml) and adding it dropwise to 500 ml hexane precipitated the raw product. The precipitate was filtered, dried and reacted for 15 min with a 1% solution of toluene sulfonic acid in dichloromethane/methanol. After the reaction the mixture was made up to a final volume of 300 ml with dichloromethane, washed with 100 ml of a saturated aqueous solution of sodium hydrogen carbonate and 100 ml water, dried over sodium sulfate and evaporated to a final volume of 20 ml. The residual solution thus purified is added dropwise into 500 ml hexane, the resulting precipitate is filtered and washed with hexane. The resulting amorphous solid is finally purified by column chromatography (silica gel, 160 g, column 6 x 15 cm, washed with 1 liter dichloromethane and then a gradient solution of dichloromethane with methanol 99:1 to 50:1 is applied. 5 Liter of this gradient eluant is collected as main fraction). The product fractions are collected and evaporated. The final product is obtained as 4.5 g foam. The overall yield is 78 mol%.

Example 2

[87] *Preparation of Thymidylyl-{3'-[O^8-(2-cyanoethyl)]-5'}-3'-O-[2-(2-nitrophenyl) propyloxycarbonyl]) thymidine*

[88] A mixture of 1 g 5'-O-Dimethoxytritylthymidine-3'-(2-cyanoethyl-N-diisopropylphosphite amide (1.06 mmol, 1 equivalent), 396 mg 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl] thymidine (0.88 mmol, 0.83 equivalent) and 350 mg tetrazole (5 mmol,) was stirred in 10 ml acetonitrile under argon protection atmosphere and light exclusion for 5 hours at room temperature. The reaction solution was stored over night at - 19 ^{0}C. The next day a 05 molar iodine solution in pyridine/dichloromethane/water (ratio 3:1:1) was added dropwise to the reaction solution until the color remained stable and then the solution was stirred for another 30 min. The reaction solution was mixed with 150 ml dichloromethane, sodium thiosulfate solution was added until decolorization occurred. The solution was dried over sodium sulfate and the solvent was evaporated. The rest was dissolved in toluene and the solvent evaporated. The rest was dissolved in 100 ml of a 1% toluene sulfonic acid solution in dichloromethane and methanol (ratio 9:1). After 15 min the solution was treated with 60 ml 1% sodium hydrogen carbonate solution, washed with 60 ml water, dried with sodium sulfate and evaporated with a rotary evaporator resulting a

solid foam. Thinlayer chromatography (hexane /ethylacetate, 1:1) confirmed that no more 3′-NPPOC-Thd was left in the product.

[89] The final purification of the product (1.2 g) was done by column chromatography (80 g silica gel, column 4x17 cm, washed with 0.5 liter dichloromethane, main fraction 3 liter using a gradient of dichloromethane/methanol 49:1 to 9:1).

[90] The main fraction was collected, evaporated and resulted in a solid amorphous foam. The yield was 65%.

Example 3

[91] *Preparation of 2-Bromoethoxy-dichlorophosphane*

[92] 12.5 g Bromoethanol (0.1 mol, 7 ml) in 10 ml acetonitrile was added to a solution of 16.5 g phosphorus trichloride (0.12 mol, 10.5 ml) in 20 ml acetonitrile at –20°C and was stirred at this temperature for 45 min. This solution was brought to –5°C while stirring within one hour and then was stirred for another hour. Subsequently the solvent was evaporated at 30 to 35 °C. The rest was dissolved in 15 ml acetonitrile and evaporated again. The final end product obtained (from acetonitrile) was 19.2 g, which corresponds to yield of 85%.

[93] The oily end product was used directly in the synthesis of Example 4 without any further purification.

Example 4

[94] *Preparation of 2-Bromoethoxy-chloro-diethylaminophosphane*

[95] 12,4 g Diethyltrimethylsilylamine (85.3 mmol, 16.1 ml) in 10 ml dichloromethane were added within one hour at about –20 °C under argon protection atmosphere to 19.1 g 2-bromoethoxy-dichlorophosphane (84,5 mmol), prepared in Example 4, in 20 ml dichloroethane. The reaction mixture was stirred over night at room temperature. Dichloromethane and the trimethylsilyl chloride formed during the reaction was evaporated at 33 °C under vacuum (2-5 mm Hg). 24g of the resulting oily yellow reaction product

(yield 99 mol%) was used for the phosphitization in Example 5, without further purification.

Example 5

[96] *Preparation of 5'-O-DMT-Thymidine-3'-O-(2-bromoethyldiethylamino phosphite)*

[97] 0,51 g Amidite from Example 4 (1.94 mmol, 1.4 equivalents) were added under argon protection atmosphere to a solution of 1 g (1.389 mmol) 5'-O-DMT-Thymidine and 0,63 g (4.86 mmol, 0.83 ml, 3.5 equivalents) diisopropylethylamine in 7 ml dichloroethane. The resulting reaction solution was stirred at room temperature for 4 hours. Subsequently another 0,39 g of the amidite from Example (total of 2.4 equivalents, 3.42 mmol) were added to the reaction solution and stirred for one hour. Then 0.2 ml methanol were added, left for 5 min, diluted with 100 ml dichloromethane, washed with 60 ml of sodium hydrogen carbonate solution and water and dried over sodium sulfate. After the solvent has been removed by evaporation, the resulting 2g of oil were finally purified by column

	B	R	R^1
1	T	iPr	EtCN
2	T	Et	NPE
3	dA^{tac}	iPr	EtCN
4	dA^{Bz}	iPr	EtCN
5	dC^{Ac}	iPr	EtCN
6	dG^{tac}	iPr	EtCN

	B^1	R
7	T	NPPOC
8	T	NPC
9	dC^{Ac}	NPPOC
10	dG^{dmf}	NPPOC

	B	B^1	R	R^2
11	T	T	EtCN	NPPOC
12	T	T	EtCN	NPC
13	T	dC^{Ac}	EtCN	NPPOC
14	T	dG^{dmf}	EtCN	NPPOC
15	T	T	NPE	NPPOC
16	dA^{tac}	T	EtCN	NPPOC
17	dA^{Bz}	T	EtCN	NPPOC
18	dC^{Ac}	T	EtCN	NPPOC
19	dG^{tac}	T	EtCN	NPPOC

chromatography (70 g silica gel, column 4x15 cm, and solvent gradient hexane/acetone (ratio 4:1) with 0,1 % triethylamine to 1:1 with 0.1 % triethylamine). The collected product fractions resulted in a yield of 6 Mol% after the solvent had been removed. The UV-spectrum in methanol showed characteristic bands at 301, 236 and 215 nm.

[98] Examples 6 to 14 of oligonucleotides according to the invention synthesized by a process according to the invention are summarized in Table 1 and described in detail in the following:

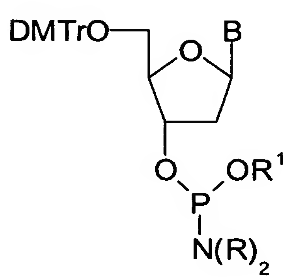
[99] **Table 1:** Starting Materials (1 –10) and synthesized oligonucleotides (11-19)

[100] Abbreviations in Table 1 represent the following groups:

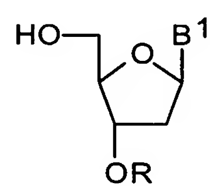
Ac	acetyl
Bz	benzoyl
d	deoxy
dmf	dimethylaminomethylene
tac	1-(4-tert-butyl)-phenoxyacetyl

[101] A, C, G, T represent adeninyl, cytosinyl, guaninyl, thyminyl respectively.

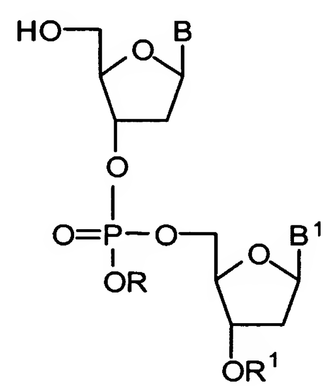
[102] Compounds 1 to 19 as mentioned in table 1 are represented by the following formulae:



compounds 1-6



compounds 7-10



compounds 11-19

Example 6

[103] *Preparation of Thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]thymidine* (**11**).

[104] A mixture of 5'-O-(4,4'-dimethoxytrityl)thymidine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**1**) (4.3 g, 5.8 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**7**) (2.0 g, 4.45 mmol) and 4,5-dicyanoimidazole (3.4 g, 29 mmol) in acetonitrile (50 ml) was stirred at room temperature (r.t.) under argon for 18 h and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (15 ml). After 20 min, the mixture was diluted with dichloromethane (400 ml), washed with saturated solution of sodium thiosulfate (2x100 ml) and then with water (1x100 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (70 ml). After 10 min, the solution was diluted with dichloromethane (200 ml), washed with a saturated solution of sodium hydrogen carbonate (2x70 ml) and then with water (1x70 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was dissolved in dichloromethane (50 ml), and the resulting solution added into n-hexane (500 ml). The precipitate was filtered off and purified by CC (silica gel, dichloromethane, dichloromethane/methanol 100:1 and then 20:1) to give 2.94 g (82%) of thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]thymidine (**11**) as a foam. UV (MeOH, λ_{max} nm (*log* ε): 263 (4.35), 210 (4.38). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.38 and 11.36 (2 *s*, 2 NH); 7.83 (*d*, H, *ortho* to NO_2); 7.71 (*m*, 2 H arom. and H-C(6)); 7.51 (*s*, H-C(6)); 7.48 (*m*, H *meta* to NO_2); 6.16 (*m*, 2 H-C(1')); 5.25 (*dd*, CH_2OH); 5.10 and 4.98 (2 *m*, 2 H-C(3')); 4.21 (*m*, H-C(4'), $CNCH_2CH_2$, $POCH_2CH$ and $COOCH_2$); 4.06 (*br.s*, H-C(4')); 3.58 (*m*, CH_2OH); 3.51 (m, CH_3CH); 2.92 (*m*, $CNCH_2$); 2.34 (*m*, 4 H-C(2')); 1.77 and 1.76 (2 *s*, 2 Me-C(5)); 1.27 (*d*, CH_3CH).

Example 7

[105] *Preparation of Thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-(4-nitrophenyloxycarbonyl)thymidine* (**12**).

[106] A mixture of 5'-O-(4,4'-dimethoxytrityl)thymidine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**1**) (0.83 g, 1.11 mmol), 3'-O-(4-nitrophenyloxycarbonyl)thymidine (**8**) (0.35 g, 0.85 mmol) and 4,5-dicyanoimidazole (0.65 g, 5.5 mmol) in acetonitrile (8 ml) was stirred at r.t. under argon for 4 h and then treated with a solution of iodine (0.4 g) in a mixture of dichloromethane/water/pyridine 1:1:3 (5 ml). After 20 min, the mixture was diluted with dichloromethane (80 ml), washed with saturated solution of sodium thiosulfate (2x30 ml) and then with phosphate buffer pH 7.0 (2x30 ml). The org. layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x15 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (5.2 ml). After 8 min, the solution was diluted with dichloromethane (80 ml), washed with a solution of sodium hydrogen carbonate (50 mg) in water (30 ml) and then with phosphate buffer pH7.0 (2x30 ml). The org. layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, dichloromethane, dichloromethane/methanol 50:1 and then 9:1) to give 0.39 g (60%) of thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-(4-nitrophenyloxycarbonyl)thymidine (**12**) as a foam. UV (MeOH, λ_{max} nm (*log* ε): 265 (4.46), 211 (4.41). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.40 and 11.34 (2 *s*, 2 NH); 8.32 (*d*, 2 H, *ortho* to NO_2); 7.66 (s, H-C(6)); 7.60 (*d*, H *meta* to NO_2); 7.56 (*s*, H-C(6)); 6.22 (*m*, 2 H-C(1')); 5.31 (*m*, H-C(3')); 5.22 (*dd*, CH_2OH); 4.99 (*m*, H-C(3')); 4.45 (*m*, H-C(4')); 4.34 (*m*, $POCH_2CH$); 4.22 (*m*, $CNCH_2CH_2$); 4.08 (*br.s*, H-C(4')); 3.59 (*m*, CH_2OH); 2.93 (*m*, $CNCH_2$); 2.49 and 2.37 (2 *m*, 4 H-C(2')); 1.79 and 1.75 (2 *s*, 2 Me-C(5)).

Example 8

[107] *Preparation of Thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-N^4-acetyl-2'-deoxy-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]cytidine* (**13**).

[108] A mixture of 5'-O-(4,4'-dimethoxytrityl)thymidine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**1**) (3.66 g, 4.91 mmol), N^4-acetyl-2'-deoxy-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)cytidine (**9**) (1.8 g, 3.78 mmol) and 4,5-dicyanoimidazole (2.23 g, 18.9 mmol) in acetonitrile (50 ml) was stirred at r.t. under nitrogen for 30 min and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (12 ml). After 20 min, the mixture was diluted with dichloromethane (500 ml), washed with saturated solution of sodium thiosulfate (2x150 ml) and then with water (1x150 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (63 ml). After 20 min, the solution was diluted with dichloromethane (500 ml), washed with a saturated solution of sodium hydrogen carbonate (2x150 ml) and then with water (1x150 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, ethylacetate, ethylacetate/methanol 100:1→ 20:1) to give 2 g (65%) of thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-N^4-acetyl-2'-deoxy-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]cytidine (**13**) as a foam. UV (MeOH, λ nm (*log* ε): 300 sh (3.80), 252 (4.32), 212 (4.37). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.33 (s, NH)); 10.91 (*s*, N*H*Ac); 8.10 (*d*, H-C(6) from Cyt); 7.84 (*d*, H *ortho* to NO_2); 7.71 (*m*, 2 H arom. and H-C(6) from Thy); 7.48 (*m*, H *meta* to NO_2); 7.22 (*d*, H-C(5) from Cyt); 6.18 and 6.07 (2 *dd*, 2 H-C(1')); 5.21 (*dd*, CH_2OH); 5.14 and 4.97 (2 *m*, 2 H-C(3')); 4.29 (*m*, H-C(4'), $POCH_2CH$ and $COOCH_2$); 4.20 (*m*, $CNCH_2CH_2$); 4.05 (*br.s*, H-C(4')); 3.58 (*m*, CH_2OH); 3.50 (m, CH_3CH); 2.92 (*m*, $CNCH_2$); 2.35 (*m*, 4 H-C(2')); 2.08 (*s*, Ac), 1.76 (*s*, Me-C(5)); 1.27 (*d*, CH_3CH).

Example 9

[109] *Preparation of Thymidylyl-{3'-[*O^P*-(2-cyanoethyl)]→5'}-2'-deoxy-*N^2*-dimethylaminomethylene-3'-*O*-[2-(2-nitrophenyl)propyloxycarbonyl]guanosine* (**14**).

[110] A mixture of 5'-O-(4,4'-dimethoxytrityl)thymidine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**1**) (6.3 g, 8.5 mmol), 2'-deoxy-N^2-dimethylaminomethylene-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)guanosine (**10**) (3 g, 5.66 mmol) and 4,5-dicyanoimidazole (5 g, 42.5 mmol) in acetonitrile (100 ml) was stirred at r.t. under

nitrogen for 30 min and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (15 ml). After 20 min, the mixture was diluted with dichloromethane (500 ml), washed with saturated solution of sodium thiosulfate (2x200 ml) and then with water (1x200 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (120 ml). After 20 min, the solution was diluted with dichloromethane (500 ml), washed with a saturated solution of sodium hydrogen carbonate (2x200 ml) and then with water (1x200 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, dichloromethane, dichloromethane/methanol 50:1 - 10:1) to give 2.24 g (45%) of thymidylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-2'-deoxy-N^4-dimethylaminomethylene-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]guanosine (**14**) as a foam. UV (MeOH, λ_{max} nm (*log* ε): 303 (4.35), 272 (4.32), 237 (4.30), 209 (4.42). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.35 (*m*, 2 NH); 8.58 (s, C*H*N(Me)$_2$); 7.97 (*s*, H-C(8) from Gua); 7.83 (*d*, H *ortho* to NO_2); 7.73 (*m*, 2 H arom. and H-C(6)); 7.51 (*m*, H *meta* to NO_2); 6.18 (*m*, 2 H-C(1')); 5.37 (*m*, CH_2O*H*); 5.18 and 4.94 (2 *m*, 2 H-C(3')); 4.34-3.98 (*m*, 2 H-C(4'), $CNCH_2CH_2$, $POCH_2$CH and $COOCH_2$); 3.52 (*m*, CH_2OH and CH_3CH); 3.16 and 3.02 (2 *s*, (Me)$_2$N); 2.88 (*m*, $CNCH_2$); 2.52 and 2.30 (2 *m*, 4 H-C(2')); 1.76 (*s*, Me-C(5)); 1.28 (*d*, CH_3CH).

Example 10

[111] *Preparation of Thymidylyl-{3'-[O^P-2-(4-nitrophenyl)ethyl]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]thymidine* (**15**).

[112] A mixture of 5'-O-(4,4'-dimethoxytrityl)thymidine-3'-O-[2-(4-nitrophenyl)ethyl-N,N-diethylphosphoramidite] (**2**) (5.6 g, 6.9 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**7**) (2.1 g, 4.67 mmol) and 4,5-dicyanoimidazole (4.1 g, 34.7 mmol) in acetonitrile (80 ml) was stirred at r.t. under argon for 18 h and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (15 ml). After 30 min, the mixture was diluted with dichloromethane (500 ml), washed with saturated solution of sodium thiosulfate (2x100 ml) and then with water (2x100 ml). The organic layer was separated, dried over anhydrous sodium sulfate and

evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (100 ml). After 15 min, the solution was diluted with dichloromethane (400 ml), washed with a saturated solution of sodium hydrogen carbonate (2x100 ml) and then with water (1x100 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was dissolved in dichloromethane (70 ml), and the resulting solution added into n-hexane (800 ml). The precipitate was filtered off and purified by CC (silica gel, dichloromethane, dichloromethane/methanol 100:1 and then 20:1) to give 2.15 g (51%) of thymidylyl-{3'-[O^P-2-(4-nitrophenyl)ethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl]thymidine (**15**) as a foam. UV (MeOH, λ_{max} nm (*log* ε): 264 (4.48), 210 (4.47). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.37 and 11.36 (2 *s*, 2 NH); 8.14 and 8.12 (2 *d*, 2 H *ortho* to NO_2 from NPE); 7.81 (*d*, H *ortho* to NO_2 from NPPOC); 7.70-7.44 (*m*, 5 H, arom. and 2 H-C(6)); 6.12 (*m*, 2 H-C(1')); 5.20 (*br.s* CH_2OH); 5.05 and 4.85 (2 *m*, 2 H-C(3')); 4.31-3.93 (*m*, 2 H-C(4'), 2x($POCH_2$) and $COOCH_2$); 3.51 (*m*, CH_2OH); 3.44 (*m*, CH_3CH); 3.07 (m, $POCH_2CH_2$); 2.27 (*m*, 4 H-C(2')); 1.75 and 1.72 (2 *s*, 2 Me-C(5)); 1.27 (*d*, CH_3CH).

Example 11

[113] *Preparation of* N^6*-[1-(4-tert-Butyl)phenyloxyacetyl)]-2'-deoxyadenylyl-{3'-[*O^P*-(2-cyanoethyl)]→5'}-3'-*O*-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine* (**16**).

[114] A mixture of N^6-[1-(4-*tert*-butyl)phenyloxyacetyl)]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)adenosine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**3**) (0.94 g, 1 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**7**) (0.36 g, 0.8 mmol) and 4,5-dicyanoimidazole (0.59 g, 5 mmol) in acetonitrile (10 ml) was stirred at r.t. under nitrogen for 1 h and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (2 ml). After 20 min, the mixture was diluted with dichloromethane (100 ml), washed with saturated solution of sodium thiosulfate (2x30 ml) and then with water (1x30 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x15 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (25 ml). After 15 min, the solution was diluted with

dichloromethane (100 ml), washed with a saturated solution of sodium hydrogen carbonate (2x30 ml) and then with water (1x30 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, dichloromethane and then with dichloromethane/methanol 50:1→ 10:1) to give 0.48 g (60%) of N^6-[1-(4-*tert*-butyl)phenyloxyacetyl)]-2'-deoxyadenylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**16**) as a foam. UV (MeOH, λ_{max} nm (*log ε*): 271 (4.42), 215 (4.45). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.39 and 11.36 (2 *s*, NH from Thy, diastereoisomers); 10.92 (s, NH from Ade); 8.70, 8.69, 8.67 and 8.65 (4 *s*, 2 H-C(2) and 2 H-C(8) from Ade, diastereoisomers); 7.82 (*d*, H *ortho* to NO_2); 7.68 (*m*, 2 H from NO_2Ph); 7.53 (*s*, H-C(6)); 7.47 (*m*, H *meta* to NO_2); 7.29 (*d*, 2 H *meta* to *t*-Bu); 6.87 (*d*, 2 H *ortho* to *t*-Bu); 6.49 and 6.14 (2 *m*, 2 H-C(1')); 5.16 (*m*, 2 H-C(3') and CH_2OH); 4.99 (s, NHCOCH_2); 4.27 (*m*, 2 H-C(4'), CNCH$_2$CH_2, POCH_2CH and COOCH$_2$); 3.60-3.10 (*m*, CH_2OH, CH$_3$C*H*, and H-C(2')); 2.95 (*m*, CNCH$_2$); 2.70 (*m*, H-C(2'); 2.33 (*m*, 2 H-C(2')); 1.78 and 1.76 (2 *s*, 2 Me-C(5)); 1.26 (*d*, CH_3CH); 1.23 (*s*, 9 H from *t*-Bu).

Example 12

[115] *Preparation of* N^6*-Benzoyl-2'- deoxyadenylyl-{3'-[*O^P*-(2-cyanoethyl)]→5'}-3'-*O-*[2-(2-nitrophenyl)propyloxycarbonyl)thymidin* (**17**).

[116] A mixture of N^6-bensoyl-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)adenosine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**4**) (4.58 g, 5.33 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**7**) (1.4 g, 3.11 mmol) and 4,5-dicyanoimidazole (3.19 g, 26.7 mmol) in acetonitrile (100 ml) was stirred at r.t. under nitrogen for 30 min and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (18 ml). After 20 min, the mixture was diluted with dichloromethane (600 ml), washed with saturated solution of sodium thiosulfate (2x250 ml) and then with water (1x250 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (170 ml). After 20 min, the solution was diluted with dichloromethane (600 ml), washed with a saturated solution of sodium hydrogen carbonate

(2x200 ml) and then with water (1x200 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, ethylacetate, ethylacetate/methanol 100:1→ 10:1) to give 1.98 g (69%) of N^6-bensoyl-2'-deoxyadenylyl-{3'-[O^P-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine **(17)** as a foam. UV (MeOH, λ_{max} nm (*log* ε): 274 (4.41), 212 (4.38). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.38 and 11.36 (2 *s*, NH from Thy, diastereoisomers); 11.22 (s, NH from Ade); 8.74, 8.73, 8.68 and 8.67 (4 *s*, 2 H-C(2) and 2 H-C(8) from Ade, diastereoisomers); 8.03 (*d*, 2 H *ortho* from Bz); 7.70-7.44 (*m*, 6 H arom. and H-C(6)); 6.52 and 6.13 (2 *m*, 2 H-C(1')); 5.22 (*m*, H-C(3') and CH_2OH); 5.14 (m, H-C(3')); 4.29 (*m*, 2 H-C(4'), $CNCH_2CH_2$, $POCH_2CH$ and $COOCH_2$); 3.60 (*m*, CH_2OH); 3.50 (*m*, CH_3CH); 3.08 (*m*, H-C(2')); 2.96 (*m*, $CNCH_2$); 2.68 (*m*, H-C(2'); 2.33 (*m*, 2 H-C(2')); 1.79 and 1.77 (2 *s*, Me-C(5), diastereoisomers); 1.26 and 1.25 (2 *d*, CH_3CH, diastereoisomers)..

Example 13

[117] *Preparation of* N^4-*Acetyl-2'- deoxycytidylyl-{3'-[*O^P*-(2-cyanoethyl)]→5'}-3'*-O-*[2-(2-nitrophenyl)propyloxycarbonyl)thymidine* **(18)**.

[118] A mixture of N^4-acetyl-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)cytidine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] **(5)** (5.58 g, 7.22 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine **(7)** (2.5 g, 5.56 mmol) and 4,5-dicyanoimidazole (3.28 g, 27.8 mmol) in acetonitrile (100 ml) was stirred at r.t. under nitrogen for 30 min and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (18 ml). After 20 min, the mixture was diluted with dichloromethane (600 ml), washed with saturated solution of sodium thiosulfate (2x250 ml) and then with water (1x250 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (170 ml). After 20 min, the solution was diluted with dichloromethane (600 ml), washed with a saturated solution of sodium hydrogen carbonate (2x200 ml) and then with water (1x200 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel,

ethylacetate, ethylacetate/methanol 100:1→ 10:1) to give 2.92 g (63%) of N[4]-acetyl-2'-deoxycytidylyl-{3'-[O[P]-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**18**) as a foam. UV (MeOH, λ_{max} nm (*log* ε): 300 sh (3.87), 250 (4.35), 212 (4.39). ^{1}H-NMR (DMSO-d_6, σ in ppm): 11.36 (s, NH)); 10.90 (*s*, N*H*Ac); 8.24 (*d*, H-C(6) from Cyt); 7.85 (*d*, H *ortho* to NO_2); 7.70 (*m*, 2 H arom.); 7.51 (*s*, H-C(6) from Thy); 7.49 (*m*, H *meta* to NO_2); 7.21 (*d*, H-C(5) from Cyt); 6.13 (*m*, 2 H-C(1')); 5.20 (*dd*, CH_2OH); 5.10 and 4.98 (2 *m*, 2 H-C(3')); 4.26 (*m*, 2 H-C(4'), $POCH_2CH$, $CNCH_2CH_2$, and $COOCH_2$); 3.60 (*m*, CH_2OH); 3.50 (m, CH_3CH); 2.93 (*m*, $CNCH_2$); 2.60 (m, H-C(2')); 2.30 (*m*, 3 H-C(2')); 2.08 (s, Ac), 1.77 (*s*, Me-C(5)); 1.27 (*d*, CH_3CH).

Example 14

[119] *Preparation of* N[2]-*[1-(4-tert-Butyl)phenyloxyacetyl)]-2'-deoxyguanylyl-{3'-[*O[P]*-(2-cyanoethyl)]→5'}-3'-*O*-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine* (**19**).

[120] A mixture of N[2]-[1-(4-*tert*-butyl)phenyloxyacetyl)]-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**6**) (7.26 g, 7.56 mmol), 3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**7**) (2 g, 4.45 mmol) and 4,5-dicyanoimidazole (4.47 g, 37.85 mmol) in acetonitrile (100 ml) was stirred at r.t. under nitrogen for 3 h and then treated with a solution of 0.5 M iodine in a mixture of dichloromethane/water/pyridine 1:1:3 (20 ml). After 20 min, the mixture was diluted with dichloromethane (500 ml), washed with saturated solution of sodium thiosulfate (2x250 ml) and then with water (1x250 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was co-evaporated with toluene (2x50 ml) and treated with a solution of 2% toluene-4-sulfonic acid in a mixture of dichloromethane/methanol 4:1 (120 ml). After 15 min, the solution was diluted with dichloromethane (500 ml), washed with a saturated solution of sodium hydrogen carbonate (2x200 ml) and then with water (1x200 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The rest was purified by CC (silica gel, ethylacetate, ethylacetate/methanol 50:1→ 10:1and then with dichloromethane/methanol 9:1) to give 2.2 g (48%) of N[2]-[1-(4-*tert*-butyl)phenyloxyacetyl)]-2'-deoxyguanylyl-{3'-[O[P]-(2-cyanoethyl)]→5'}-3'-O-[2-(2-nitrophenyl)propyloxycarbonyl)thymidine (**19**) as a foam. UV (MeOH, λ nm (*log* ε): 277 sh (4.25), 260 (4.38), 210 (4.44). ^{1}H-NMR (DMSO-

d_6, σ in ppm): 11.80 (s, H-N(1) and NH-C(2) from Gua); 11.39 (*s*, NH from Thy); 8.27 (*s*, H-C(8) from Gua); 7.80 (*m*, H *ortho* to NO_2); 7.66 (*m*, 2 H arom. from NO_2Ph); and)); 7.48 (*m*, H-C(6) and H *meta* to NO_2); 7.30 (*d*, 2 H *meta* to *t*-Bu); 6.87 (*d*, 2 H *ortho* to *t*-Bu); 6.23 (*m*, 2 H-C(1')); 5.16 (*m*, CH_2OH); 5.15 and 5.10 (2 *m*, 2 H-C(3')); 4.82 (s, $NHCOCH_2$); 4.27 (*m*, 2 H-C(4'), $CNCH_2CH_2$, $POCH_2CH$ and $COOCH_2$); 3.58 (*m*, CH_2OH); 3.46 (*m*, CH_3CH); 2.95 (*m*, $CNCH_2$ and H-C(2')); 2.67 (m, H-C(2')); 2.41 (*m*, 2 H-C(2')); 1.70 (*s*, Me-C(5)); 1.28 (*m*, CH_3CH and 9 H from *t*-Bu).

Example 15

[121] *General procedure (A) for the synthesis of 5'-0-Dimethoxytrityl-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxynucleosides (20, 21)*

[122] A solution of 420 mg (1.6 mmol) (9-fluorenylmethyl)chloroformiat in 4 ml anhydrous dichloromethanewas dropped to 1 of mmol 5'-0-dimethoxytrityl-2'-deoxy-nucleoside (coevapurated with pyridine in 4 ml anhydrous pyridine. After stirring 4 h at r.t. the mixture was diluted with 15 ml H_2O and extracted with CH_2Cl_2. The organic phase was dried out $MgS0_4$, filtered, and evaporated. The crude product was purified by CC (silica gel, PE/EE 3:1 to 1:2) to give the desired products (**20, 21**).

Example 16

[123] *Preparation of 5'-0-Dimethoxytrityl-3'-0-(9-fluorenylmethoxycarbonyl)-thymidine (20)*

[124] Compound 20 was prepared in 89 % yiel following the general procedure A using 3.57 g (13.8 mmol) (p-fluorenylmethyl)chloroformiat/15 ml anhydrous dichloromethane and 4.42 g (8.1 mmol) 5'-0-dimethoxytritylthymidine/15 ml anhydrous pyridine.
UV (MeOH), λ [nm]: 204 (4.99), [216 (4.62)]. [234 (4.39)], [255 (4.42)]. 263 (4.48), [268 (4.41)], [285 (3.90)]. [298 (3.75)]; ^{1}H-NMR (250 MHz, $CDCl_3$): 8.37 (s, 1H, NH). 7.75 (d, 2H, 2 x arom. H FMOC), 7.59 (m, 3H, 2 x arom. H FMOC, H-C(6)), 7.43-7.23 (m, 13H. 4 x arom. H FMOC, 9 x arom. H DMTr), 6.81 (d, 4H, 4 x arom. H DMTr), 6.47 (m, 1H, H-C(l')), 5.34 (m. 1H, H-C(3')), 4.41 (m, 2H, CHa FMOC), 4.23 (m. 2H, H-C(4'), H-C(9) FMOC), 3.76 (s, 6H, 2 x OCH_3

DMTr), 3.47 (m, 2H. 2 x H-C(5')), 2.51-2.42 (m, 2H, 2 x H-C(2')), 1.36 (s, 3H, CH_3 Thy); Anal. calcd. for $C_{46}H_{42}N_20_9$ x 0.5 H_2O (775.87). C 71.21, H 5.59, N 3.61; found: C 71.07, H 5.74, N 3.55.

Example 17

[125] *Preparation of N^4-Benzoyl-5'-O-dimethoxytrityl-3'-O-(9-fluorenylmethoxy-carbonyl)-2'-deoxycytidine (21)*

[126] Compound 21 was prepared in 87 % yield following the general procedure A using 4.4 g (17 mmol) (9-fluorenylmethyl)chloroformiat/20 ml anhydrous dichloro-methane and 6.34 g (10 mmol) N^4-benzoyl-5'-O-dimethoxytrityl-2'-deoxycytidine/ 20 ml anhydrous pyridine.

[127] UV (MeOH), λ_{max} [nm]: 204 (5.03). [216 (4.69)], 236 (4.55), 260 (4.62), [268 (4.52)], 298 (4.19), [309 (4.01)]; ^{1}H-NMR (250 MHz, $CDCl_3$): 8.79 (s, 1H, NH), 8.15 (d, 1H, H-C(5)), 7.88 (d, 2H. 2 x arom. H FMOC), 7.75 (d, 2H, 2 x arom. H Bz), 7.60 (d, 2H, 2 x arom. H FMOC), 7.61-7.21 (m, 17H, 4 x arom. H FMOC, H-C(6), 9 x arom. H DMTr, 3 x arom. H Bz), 6.83 (dd, 4H, 4 x arom. H DMTr), 6.34 (m, 1H, H-C(l')). 5.31 (m, 1H, H-C(3')), 4.43 (m, 2H, CH_2 FMOC), 4.35 (m, 1H, H C(4')), 4.25 (t, 1H, H-C(9) FMOC), 3.76 + 3.75 (2 x s, 6H, 2 x OCH_3 DMTr), 3.48 (m, 2H, 2 x H-C(5')), 2.91 (m, 1H. H-C(2')), 2.34 (m, 1H, H-C(2')); Anal. calcd. for $C_{52}H_{47}N_3O_9$ x 0.5 H_2O (866.98). C 72.04, H 5.58, N 4.84; found: C 71.62. H 5.43, N 4.80.

Example 18

[128] *General procedure (B) for the synthesis of 3'-0-(9-Fluorenylmethoxycarbonyl)-2'-deoxynucleosides (22, 23)*

[129] 1 mmol 5'-0-Dimethoxytrityl-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxynucle-oside (20, 21) was dissolved in 10 ml of a 2 % solution of toluene-4-sulfonic acid in dichloromethane/methanol 4:1. After stirring at r.t. for l h the mixture was diluted with 15 ml H_2O, and extracted twice with CH_2Cl_2. The organic phase was dried over $MgS0_4$,

filtered, and evaporated. The crude product was purified by CC (silica gel, CH_2Cl_2/MeOH, 100:0 to 100:3.5) to give the desired products (22, 23).

Example 19

[130] *Preparation of 3'-0-(9-Fluorenylmethoxycarbonyl)-thymidine (22)*

[131] Compound 22 was prepared in 95 % yield following the general procedure B using 450 mg (0.59 mmol) 5'-0-dimethoxytrityl-3'-0-(9-fluorenylmethoxycarbonyl)-thymidine (1)/5.9 ml of 2 % toluene-4-sulfonic acid-solution.

UV (MeOH), λ_{max} [nm]: 206 (4.72), [218 (4.35)], [224 (4.02)]. [256 (4.40)], 263 (4.46), [268 (4.39)], [285 (3.83)], 298 (3.73); ^{1}H-NMR (250 MHz, $CDCl_3$): 8.27 (s, 1H, NH), 7.76 (d. 2H, 2 x arom. H FMOC), 7.59 (d, 2H, 2 x arom. H FMOC). 7.44-7.26 (m, 5H, 4 x arom. H FMOC, H-C(6)), 6.19 (m, 1H. H-C(l')). 5.26 (m, 1H, H-C(3')), 4.45 (d, 2H, CH_2 FMOC), 4.24 (t, 1H, H-C(9) FMOC), 4.15 (m, 1H, H-C(4')), 3.88 (m, 2H. 2 x H-C(5')), 2.47 (m, 3H, OH-C(5'), 2 x H-C(2')), 1.91 (s, 3H, CH_3 Thy); Anal. calcd. for $C_{25}H_{24}N_2O_7$ x 0.5 H_2O (473.49). C 63.42, H 5.32, N 5.92; found: C 63.38, H 5.24. N 5.81.

Example 20

[132] *Preparation of N^4-Benzoyl-3'-O-(9-fluorenylmethoxycarbonyl)-2'-deoxycytidine (23)*

[133] Compound **23** was prepared in 78 % yield following the general procedure **B** using 7.23 g (8.4 mmol) N^4-benzoyl-5'-O-dimethoxytrityl-3'-O-(9-fluorenylmethoxycarbonyl)-2'-deoxycytidine (2)/84 ml of 2 % toluene-4-sulfonic acid-solution.

[134] UV (MeOH), λ_{max} [nm]: 205 (4.80), [216 (4.49)], [224 (4.23)], [256 (4.61)]. 260 (4.63), [268 (4.52)], [288 (4.13)], 298 (4.20), [306 (4.04)]; ^{1}H-NMR (250 **MHz, DMSO-d_6):** 11.27 (s(br), 1H, NH), 8.34 (d, 1H, H-C(5)), 7.99 (d, 2H, 2 x arom. H FMOC), 7.90 (d, 2H, 2 x arom. H Bz), 7.68-7.32 (m, 10H, 6 x arom. H FMOC, H-C(6), 3 x arom. H Bz), 6.10 (m, 1H, H-C(l')), 5.21 (t, 1H, OH-C(5')), 5.11 (m, 1H, H-C(3')), 4.60 (d, 2H, CH_2 FMOC), 4.33 (t, 1H, H-C(9) FMOC), 4.11 (m, 1H, H-C(4')), 3.62 (m. 2H, 2 x H-C(5')), 2.43 (m, 1H, H-C(2')), 2.26 (m, 1H, H-C(2'));

Anal. calcd. for $C_{31}H_{27}N_3O_7$ x 0.5 H_2O(553.58). C 67.26, H 4.92, N 7.59; found: C 66.83, H 4.83, N 7.51.

Example 21

[135] *General procedure (C) for the synthesis of 5'-O-Dimethoxytrityl-2'deoxynucleoside-{3'(O^P cyanoethyl)-5'}-3'-O-(9-fluorenyl-methoxycarbonyl)-2'-deoxynucleoside **(24, 25)***

[136] To a solution of l mmol 3'-0- (9-fluorenylmethoxycarbonyl)-2'-deoxynucleoside (22, 23) and 1.7 mmol 5'-0-dimethoxytrityl-2'-deoxynucleosid-3'-0-[(2-cyano-ethyl)(N,N-diisopropylamino)]phosphitamide in 15 ml anhydrous acetonitrile was added under argaon atmosphere 4.4 mmol of 1H tetrazole. After stirring for 3 h at r.t. was added a mixture of oxidizing solution (500 mg iodinein 5 ml pyri-dine/water/dichloromethane 3/1/1) until iodine colour persisted. After 1 h the mixture was diluted with 40 ml dichloromethane, and extracted twice with 40 ml of a saturated solution of sodium thiosulfate. The aqueous washings were combined and re-extracted with 40 ml dichloromethane. The organic phase was dried over $MgS0_4$, filtered and evaporated. The crude product was purified by CC (silica gel CH_2Cl_2/MeOH, 100:0 to 100:3.5) to give the desired products (24, 25).

Example 22

[137] *Preparation of N^6-Benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenylyl-{3'-(O^P-cyanothyl)-5'-}-3'-0-(9-fluorenylmethoxycarbonyl)-thymidine* **(24)**

[138] Compound 24 was prepared in 90 % yield following the general procedure **C** using 465 mg (1 mmol) 3'-0-(9-fluorenylmethoxycarbonyl)-thymidine (22), 1.4 g (1.7 mmol) N^6-benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenosine-3'-O-[(2-cyano-ethyl)(N,N-diisopropylammo)]phosphitamide, 280 mg (4 mmol) tetrazole/15 ml an-hydrous acetonitrile.

[139] UV (MeOH), λ_{max} [nm]: 204 (5.09), [217 (4.77)], [224 (4.64)], [233 (4.59)], 264 (4.61), [271 (4.60)], [284 (4.42)], [295 (4.15)], [320 (3.32)]; ^{1}H-NMR (250 MHz, **$CDCl_3$):** 9.12 + 9.08 + 8.86 (3 x s, 2H. NH T/dA), 8.68 + 8.12 (2 x 2s, 2H,

H-C(2), H-C(8) dA). 8.01 (d, 2H, 2 x arom. H FMOC), 7.74 (d, 2H, 2 x arom. H FMOC). 7.61-7.16 (m, 19H, H-C(6) T, 4 x arom. H FMOC, 9 x arom. H DMTr, 5 x arom. H Bz), 6.78 (m. 4H. 4 x arom. H DMTr), 6.48 (m, 1H, H-C(l')), 6.25 (m, 1H, H-C(l')), 5.29 (m. 1H, H-C(3')), 5.24 (m, 1H, H-C(3')), 4.44-4.19 (m, 9H, CH_2 FMOC, 2 x H-C(5'), H-C(9) FMOC, 2 x H-C(4'), α-CH_2 CE), 3.74 + 3.73 (2 x s, 6H, 2 x OCH_3 DMTr), 3.42 (m, 2H, 2 x H-C(5')), 3.12 (m. 1H, H-C(2')), 2.82-2.61 (m, 3H, H-C(2'), β-CH_2 CE), 2.58-2.29 (m, 2H, 2 x H-C(2')), 1.89 (2 x s, 3H, CH_3 T); ^{31}P-NMR (400 MHz, $CDCl^3$): 1.12.

Example 23

[140] *Preparation of 5'-O-Dimethoxytrityl-thymidylyl-{3'-(O^P-cyanoethyl)5'-}-N^4-benzoyl-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxycytidine (25)*

[141] Compound 25 was prepared in 96 % yield following the general procedure **C** using 1.38 g (2.5 mmol) N^4-benzoyl-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxycytidine (23), 3.02 g (4.25 mmol) 5'-0-dimethoxytrityl-thymidine-3'-0-[(2-cyanoethyl)-(N,N-diisopropylamino)]phosphitamide, 790 mg (11.25 mmol) tetrazole/30 ml anhydrous acetonitrile.

[142] UV (MeOH), λ_{max} [nm]: 204 (5.08), [217 (4.73)], [236 (4.57)], 262 (4.68), [282 (4.46)], 298 (4.15), [305 (3.99)]; ^{1}H-NMR (250 MHz, $CDCl_3$): 8.85 (s, 1H, NH T oder dC), 8.51 + 8.47 (2 x s(br). 1H, NH T oder dC), 8.03 (2 x d. 1H, H-C(5) dC), 7.87 (m. 2H, 2 x arom. H FMOC), 7.75 (d, 2H, 2 x arom. H FMOC), 7.62-7.17 (m, 20H, H-C(6) T. H-C(6) dC, 4 x arom. H FMOC, 9 x arom. H DMTr, 5 x arom. H Bz), 6.80 (m, 4H, 4 x arom. H DMTr), 6.36 (m, 1H, H-C(l')), 6.28 (m, 1H, H-C(l')), 5.18 (m, 2H. 2 x H-C(3')), 4.41-4.12 (m, 9H, CH_2 FMOC, 2 x H-C(5'), H-C(9) FMOC, 2 x H-C(4'), α-CH_2 CE), 3.75 + 3.74 (2 x s, 6H, 2 x OCH_3 DMTr), 3.51 (m, 1H, H-C(5')), 3.37 (m, 1H, H-C(5')), 2.85 (m, 1H, H-C(2')), 2.73 (m, 1H, H-C(2')), 2.63 (m, 2H, β-CH_2 CE). 2.44 (m, 1H, H-C(2')), 2.20 (m, 1H, H-C(2')), 1.39 + 1.38 (2 x s, 3H, CH_3 T); ^{31}P-NMR (400 MHz, $CDCl_3$): 1.46 + 1.32;

Anal. calcd. for $C_{65}H_{61}N_6O_{16}P$ x H_2O (1231.23). C 63.41, H 5.16. N 6.83; found: C 63.28, H 5.16, N 6.63.

Example 24

[143] *General procedure (D) for the synthesis of 2'-Deoxynucleoside-{3'-(O^P-cyanoethyl) - 5'-}-3'-0-(9-fluorenylmethoxy-carbonyl)-2'-deoxynucleoside (26, 27)*

[144] 1 mmol 5'-Dimethoxytrityl-2'-deoxynucleoside-{3'-(O^P-cyanoethyl)-5'-}-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxynucleoside (24, 25) was dissolved in 10 ml of a 2 % solution of toluene-4-sulfonic acid in dichloromethane/methanol 4:1. After stirring at r.t. for 30 min the mixture was diluted with 15 ml HaO, and extracted twice with CHaCla. The organic phase was dried over MgS04, filtered, and evaporated. The crude product was purified by CC (silica gel, CHzCla/MeOH, 100:0 to 100:5) to give the desired products (26, 27).

Example 25

[145] *Preparation of N^6-Benzoyl-2'-deoxyadenylyl-{3'-(O^P-cyanoethyl)-5'-}-3'-0-(9-fluorenyl-*
methoxycarbonyl)-thymidine (26)

[146] Compound 26 was prepared in 79 % yield following the general procedure **D** using 1.87g (1.5 mmol) N^6-benzoyl-5'-O-dimethoxytrityl-2'-deoxyadenylyl-{3'-(O^P-cyanoethyl)-5'-}-3'-0-(9-fluorenylmethoxycarbonyl)-thymidine (24)/15 ml 2 % toluene-4-sulfonic acid-solution.

[147] UV (MeOH), λ_{max} [nm]: 205 (4.87), [217 (4.57)], [225 (4.34)], 264 (4.60), [270 (4.58)], [283 (4.41)], [295 (4.14)], [322 (3.08)]; ^{1}H-NMR (250 MHz, $CDCl_3$): 9.82 + 9.72 + 9.43 (3 x s, 1H, NH T, dA), 8.75 + 8.15 (2 x 2s, 2H. H-C(2), H-C(8) dA), 7.99 (d, 2H, 2 x arom. H FMOC), 7.74 (d, 2H, 2 x arom. H FMOC), 7.59-7.25 (m, 10H, H-C(6) T, 4 x arom. H FMOC, 5 x arom. H Bz). 6.37 (m. 1H, H-C(l')), 6.21 (m, 1H, H-C(l')). 5.92 (d(br).1H, OH-C(5')), 5.33 (m. 1H, H-C(3')), 5.22 (m, 1H, H-C(3')). 4.46-4.20 (m, 9H, CH_2 FMOC, 2 x H-C(5'), H-C(9) FMOC, 2 x

H-C(4') α-CH_2 CE), 3.87 (m, 2H, 2 x H-C(5')), 3.14 (m, 1H, H-C(2')), 2.77 (m, 2H, /3-CHa CE), 2.62 (m, 1H, H-C(2')), 2.41 (m, 2H, 2 x H-C(2')), 1.89 + 1.88 (2 x s, 3H, CH3 T); ^{31}P-NMR (400 MHz, $CDCl_3$): 1.15 + 0.93; Anal. calcd. for $C_{45}H_{43}N_8O_{13}P$ x 2 H_2O (970898). C 55.67, H 4.88, N 11.54; found: C 55.37, H 4.67, N 11.27.

Example 26

[148] *Preparation of Thymidylyl-{3'-(O^P-cyanoethyl-5'-}-N^4-benzoyl-3'-O-(9-fluorenyl-methoxycarbonyl)-2'-deoxycytidine (27)*

[149] Compound 27 was prepared in 84 % yield following the general procedure **D** using 1 g (0.82 mmol) 5'-0-dimethoxytrityl-thymidylyl- {3'-(O^P-cyanoethyl)-5' }-N^4-benzoyl-3'-0-(9-fluorenylmethoxycarbonyl)-2'-deoxycytidine (25)/9 ml 2 % toluene-4-sulfonic acid-solution.

[150] UV (MeOH), λ_{max} [nm]: 206 (4.82), [217 (4.54)], [223 (4.32)], 261 (4.69), [269 (4.60)], [286 (4.18)], 298 (4.17), [307 (4.00)]; ^{1}H-NMR (250 MHz, $CDCl_3$): 9.15 + 8.87 + 8.84 (3 x s. 1H, NH T, dC), 8.08 (m, 1H, H-C(5) dC), 7.90 (dd, 2H, 2 x arom. H FMOC), 7.75 (d, 2H, 2 x arom. H FMOC), 7.63-7.29 (m, 11H, H-C(6) T, H-C(6) dC. 4 x arom. H FMOC, 5 x arom. H Bz), 6.27 (m, 1H, H-C(l')), 6.11 (m, 1H. H-C(l')), 5.24 (m, 2H, 2 x H-C(3')), 4.44-4.20 (m, 9H, CH_2 FMOC, 2 x H-C(5'), H-C(9) FMOC, 2 x H-C(4'), α-CH_2 CE). 3.85 (m. 2H, 2 x H-C(5')), 3.32 (s(br), 1H, OH-C(5')), 2.80 (m. 3H, H-C(2'), β-CH2 CE), 2.53 (m, 2H, 2 x H-C(2')), 2.31 (m, 1H, H-C(2')), 1.88 (s, 3H, CH_3 T) ^{31}P-NMR (400 MHz, CDCl3): 1.44 + 1.39; Anal. calcd. for $C_{44}H_{43}N_6O_{14}P$ x 2 H_2O (946878). C 55.81, H 5.00, N 8.88; found: C 55.45, H 4.76, N 8.84.